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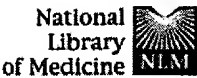
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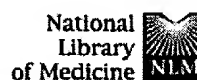
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☐ 1: Viral Immunol. 1998;11(1):27-36.

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## **A vector DNA vaccine encoding pseudorabies virus immediate early protein demonstrates partial protection in mice against lethal virus challenge.**

**Chang SW, Bu J, Rompato G, Garmendia AE.**

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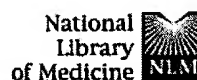
Department of Pathobiology, University of Connecticut, Storrs 06269, USA.

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An earlier study in our laboratory provided evidence that pseudorabies virus (PrV) immediate early protein (IE180) may contribute to the overall immune response against PrV. To examine the response by IE180 more closely, we initiated a vaccine trial in mice with a vector DNA construct that contains the gene encoding for IE180, designated pcDNAIE180. The DNA vaccine was delivered in gold microcarriers using a Helios Gene Gun, and 70% of BALB/c mice given the DNA vaccine (2 microg/mouse) seroconverted within 2 weeks. The remaining negative mice seroconverted after a single vaccine booster. Essentially similar results were obtained on vaccination of C57BL/6 mice, whereas C3H/HeJ mice remained negative after the first vaccination, but responded after a booster. Seven months after immunization with pcDNAIE180, an overall 25% of BALB/c, C3H/HeJ, and C57BL/6 mice receiving a lethal PrV challenge were protected. In addition, a significant passive transfer of IE180-specific antibodies to the offspring from pregnant mice vaccinated with pcDNAIE180 was observed. Interestingly, a moderate level of protection (27.6%) was also observed when these offspring received a lethal PrV challenge. Moreover, an enhancement of immune responses and a twofold increase in the level of protection were observed in mice that received a second vaccine booster by gene gun 8 months after the first vaccination. Together, these data support the theory that IE180 may indeed play a role in the overall protective immunity against PrV.

PMID: 9586695 [PubMed - indexed for MEDLINE]

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☐ 1: Vaccine. 1999 Jun 4;17(20-21):2628-35.[Related Articles, Links](#)Entrez  
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## Protection of turkeys against *Chlamydia psittaci* challenge by gene gun-based DNA immunizations.

Vanrompay D, Cox E, Vandebussche F, Volckaert G, Goddeeris B.

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Catholic University of Leuven, Laboratory of Gene Technology, Heverlee, Belgium. daisy.vanrompay@agr.kuleuven.ac.be

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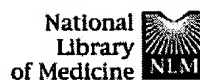
Particle-mediated (Helios Gene Gun) transfer to the turkey epidermis of plasmid DNA expressing the major outer membrane protein (MOMP) of an avian *Chlamydia psittaci* strain was evaluated for its ability to raise an immune response and protection against challenge with the homologous strain. In turkeys, the delivery of pcDNA1/MOMP coated onto 0.6 microm gold beads was the most efficient compared to immunisations using 1.0 or 1.6 microm gold beads. The delivery of as little as 1 microg pcDNA1/MOMP coated onto 0.6 microm gold beads was efficient. Immunisation with 1.0 microm gold beads required twice more (2 microg) DNA to achieve comparable results. The use of 2 microg DNA coated onto 1.6 microm gold beads had no effects. The gene gun delivery both primed T-helper and B-cell memory although recombinant MOMP-expressing cells did not induce high-titre antibody responses. The significance of gene gun-based DNA immunisation as a means of preventing severe clinical signs, lesions and chlamydia excretion in a turkey model of *Chlamydia psittaci* infection was demonstrated.

PMID: 10418912 [PubMed - indexed for MEDLINE]

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☐ 1: Vaccine. 1997 Apr-May;15(6-7):664-71.

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Erratum in:

- Vaccine 1998 Apr;16(6):655.

## **Intracutaneous vaccination of rabbits with the cottontail rabbit papillomavirus (CRPV) L1 gene protects against virus challenge .**

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**Sundaram P, Tigelaar RE, Brandsma JL.**

Section of Comparative Medicine, Yale University School of Medicine, New Haven, CT 06520, USA.

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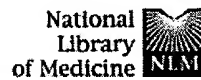
A DNA vaccine encoding the major capsid protein L1 of cottontail rabbit papillomavirus (CRPV) was constructed and administered intracutaneously (i.c.) to rabbits as supercoiled plasmids bound to gold beads using a specialized delivery device ("gene gun"). L1 DNA-vaccinated rabbits developed cellular proliferative responses to CRPV virus-like particles and developed high titered antibodies with neutralizing activity to CRPV. Following experimental challenge with CRPV, all of the L1 DNA-vaccinated rabbits, vs none of the controls, were protected from papilloma formation. These results demonstrate that i.c. vaccination of rabbits with the L1 papillomavirus capsid gene can induce antibodies that protect against subsequent papillomavirus infection.

PMID: 9178468 [PubMed - indexed for MEDLINE]

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☐ 1: Vaccine. 1999 Mar 5;17(9-10):1109-16.

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## Immunization of dogs and cats with a DNA vaccine against rabies virus.

Osorio JE, Tomlinson CC, Frank RS, Haanes EJ, Rushlow K, Haynes JR, Stinchcomb DT.

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Heska Corporation, Ft. Collins, CO 80525, USA. osorioj@heska.com

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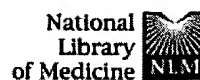
The applicability of DNA immunization technology for vaccine development in companion animals was investigated by immunizing dogs and cats by the intramuscular (i.m.) and intradermal (i.d.) routes with a plasmid DNA vector encoding the rabies virus glycoprotein G. In dogs, administration of 100 microg DNA doses by the i.m. route resulted in stronger and more durable rabies virus neutralizing antibody (RVNA) titers than those obtained by i.d. inoculation. In contrast, i.m. vaccination of cats with a similar dose was less effective in terms of mean titer and seroconversion frequency. However, efficacy was improved by increasing the dosage to 300 microg of DNA per immunization. Interestingly, i.d. inoculation of cats appeared to be a superior route of delivery in this species, resulting in higher seroconversion frequency than i.m. administration. In addition, geometric mean RVNA titers in i.d. inoculated cats increased over four-fold during a seven month period following a second and final immunization. These results demonstrate that non-facilitated, naked DNA vaccines can elicit strong, antigen-specific immune responses in dogs and cats, and DNA immunization may be a useful tool for future development of novel vaccines for these species.

PMID: 10195621 [PubMed - indexed for MEDLINE]

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☐ 1: Vaccine. 1998 Jan-Feb;16(2-3):115-8.

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## Gene gun particle-mediated vaccination with plasmid DNA confers protective immunity against rabies virus infection.

Lodmell DL, Ray NB, Ewalt LC.

Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, MT 59840, USA.

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Accell gene gun particle-mediated immunization with DNA encoding the glycoprotein gene of the challenge virus standard strain of rabies virus was evaluated for its ability to elicit protective levels of serum anti-rabies virus neutralizing antibody. Strong primary and booster neutralizing antibody responses were detected in mice following immunization with 2 micrograms of DNA coated on 2.6-micron gold beads. Protective levels of antibody persisted for over 300 days. Mice challenged intraplantarly 315 days post-primary immunization (225 days post-booster vaccination) survived lethal rabies virus challenge. Our data demonstrate a potentially significant role for gene gun-based delivery of DNA in the field of rabies virus vaccination.

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PMID: 9607018 [PubMed - indexed for MEDLINE]

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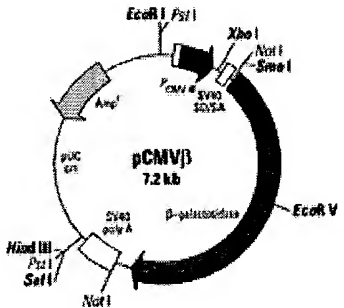
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pCMVβ



Restriction map of pCMVbeta. Unique restriction sites are in bold.

**Note:** The vector sequence file has been compiled from information in the sequence database, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.

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Description

TOP

pCMVbeta is a mammalian reporter vector designed to express beta-galactosidase in mammalian cells from the human cytomegalovirus immediate early gene promoter (1). pCMVbeta contains an intron (splice donor/splice acceptor; 2) and polyadenylation signal from SV40, and the full-length *E. coli* beta-galactosidase gene with eukaryotic translation initiation signals (3). pCMVbeta expresses high levels of beta-galactosidase and can be used as a reference (control) plasmid when transfecting other reporter gene constructs and can be used to optimize transfection protocols by employing standard assays or stains to assay beta-galactosidase activity. Alternatively, the beta-galactosidase gene can be excised using the *Not* I sites at each end to allow other genes to be inserted into the pCMVbeta vector backbone for expression in mammalian cells or to insert the beta-galactosidase fragment into another expression vector.

Vector	Size	Cat. #	GenBank Accession #
pCMVβ	20 μg	6177-1	U02451

## Location of Features



- Immediate early cytomegalovirus promoter (P<sub>CMV IE</sub>)
  - Enhancer region: 27-431
  - TATA box: 520-526
  - Transcription start point: 549
- Intron (SV40 splice donor/splice acceptor)
  - SV40 late 19s mRNA intron: 672-702
  - Modified SV40 late 16s mRNA intron (2): 672-768
- beta-galactosidase gene with eukaryotic initiation signals (3)
  - Eukaryotic translation initiation signal: 867-876
  - beta-galactosidase fusion protein:
    - start codon (ATG): 873-875; stop codon: 4014-4016
    - Amino acids from *D. melanogaster* alcohol dehydrogenase: 873-965
    - Amino acids from *E. coli* beta-galactosidase: 969-4013
    - C->A (Phe->Leu) mutation removing *EcoR* I site: 3965
- SV40 polyadenylation signal
  - Polyadenylation signal: 4426-4431
  - mRNA 3' end: 4445
- pUC origin of replication: 4918-5561
- Ampicillin resistance (beta-lactamase) gene
  - Promoter: -35 region: 6639-6634; -10 region: 6616-6611
  - Transcription start point: 6604
  - Ribosome binding site: 6581-6577
  - beta-lactamase coding sequences:
    - start codon (ATG): 6569-6567; stop codon: 5711-5709
    - beta-lactamase signal peptide: 6569-6501
    - beta-lactamase mature polypeptide: 6500-5712

Propagation in *E. coli*

- Suitable host strains: DH5 $\alpha$ , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

## References



1. MacGregor, G. R. & Caskey, C. T. (1989) Construction of plasmids that express *E. coli* beta-galactosidase in mammalian cells. *Nucleic Acids Res.* **17**:2365.
2. Okayama, H. & Berg, P. (1983) A cDNA cloning vector that permits expression of cDNA inserts in mammalian cells. *Mol. Cell Biol.* **3**:280-289.
3. MacGregor, G. R., et al. (1987) Histochemical staining of clonal mammalian cell lines expressing *E. coli* beta-galactosidase indicates heterogeneous expression of the bacterial gene. *Somat. Cell Mol. Genet.* **13**:253-265.

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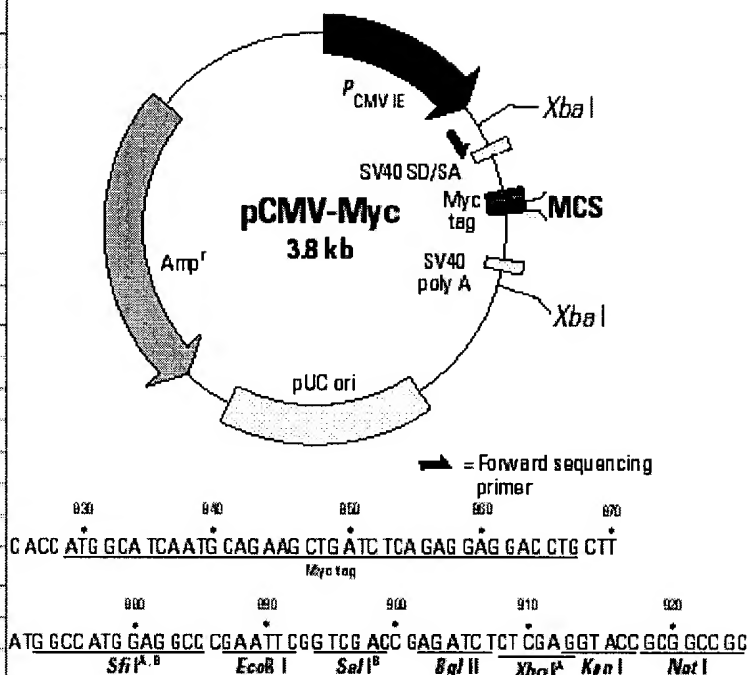
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## pCMV-Myc



**Restriction Map and Multiple Cloning Site (MCS) of pCMV-Myc.** Unique restriction sites are in bold.

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<sup>B</sup>Sites are compatible with MATCHMAKER System 3 BD Vector.

For older MATCHMAKER Systems, consult the Vector Information Packets provided with the vectors to determine compatibility.

**Note:** The vector sequence file has been compiled from information in the sequence database, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.

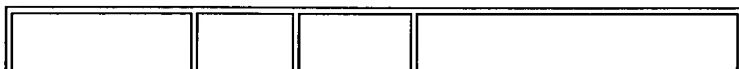
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## Description



The pCMV-Myc Mammalian Expression Vector expresses proteins containing the N-terminal c-Myc epitope tag. The c-Myc epitope tag is well-characterized and highly immunoreactive. High-level expression in mammalian cells is driven from the human cytomegalovirus immediate early promoter/enhancer ( $P_{CMVIE}$ ). The vector contains an intron (splice donor/splice acceptor); the epitope tag; an MCS; and a polyadenylation signal from SV40. This vector also possesses the ampicillin resistance gene for selection in *E. coli*.



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Vector	Size	Cat. #	GenBank Accession #
pCMV-Myc & pCMV-HA Vector Set	20 µg	K6003-1	submission in progress

## Use



To create a fusion of a gene of interest and the Myc tag, insert the gene into the MCS in frame with the Myc coding sequence. The resulting Myc-tagged proteins can be identified with the c-Myc Monoclonal Antibody, provided with this vector, or another antibody raised against the Myc tag. The epitope tag is also useful for facilitating purification of the protein, identifying associated proteins, characterizing new proteins by immunoprecipitation, and determining subcellular localization.

The MCS in this vector is compatible with the MCSs in CLONTECH's MATCHMAKER Two-Hybrid System Vectors. Compatibility with System 3 Vectors is noted in the MCS diagram. Consult the Vector Information Packet provided with any MATCHMAKER vector for complete information.

After obtaining putative positive clones in your MATCHMAKER two-hybrid screen, use the pCMV-Myc and pCMV-HA Vectors to verify the interactions identified in yeast directly in mammalian cells. To accomplish this, subclone the selected inserts into the pCMV-Myc Vector and the "bait" insert into the pCMV-HA Vector. Alternatively, clone the "bait" insert into pCMV-Myc and the library inserts into pCMV-HA. To confirm predicted interactions *in vivo* via coimmunoprecipitation, cotransfect pCMV-Myc with the pCMV-HA Vector into mammalian cells and immunoprecipitate using the c-Myc Monoclonal or HA-Tag Polyclonal Antibody provided with the vectors.

## Location of Features



- Immediate early cytomegalovirus promoter (P<sub>CMV IE</sub>):  
Enhancer region: 27–431  
TATA Box: 520–526  
Transcription start point: 549
- Intron (SV40 splice donor/splice acceptor):  
SV40 late 19s mRNA intron: 672–702  
Modified SV40 late 16s mRNA intron: 672–768
- Myc epitope tag with start codon (ATG): 829–867
- Multiple Cloning Site: 881–921
- SV40 polyadenylation signal:  
Polyadenylation signal: 1053–1058  
mRNA 3' end: 1072
- pUC plasmid replication region: 1545–2188
- Ampicillin resistance (β-lactamase) gene:  
Promoter:  
– 35 region: 3266–3261

- 10 region: 3243–3238  
Transcription start point: 3231  
Ribosome binding site: 3208–3204  
 $\beta$ -lactamase coding sequences:  
    Start codon (ATG): 3196–3194  
    Stop codon (TAA): 2338–2336  
 $\beta$ -lactamase signal peptide: 3196–3188  
 $\beta$ -lactamase mature polypeptide: 3127–2339

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#### Primer Locations



- pCMV Forward Sequencing Primer: 631–657  
5'-GAT-CCG-GTA-CTA-GAG-GAA-CTG-AAA-AAC-3'

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#### Propagation in *E. coli*



- Suitable host strains: DH5 $\alpha$ , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100  $\mu$ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500



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# pSL301

- 
- **Vector IG Sequence Link :**
  - **General :** phagemid ds-DNA 3264 BP
  - **Functions :** (cloning)
  - **Selection :** (color blue/white)
  - **Copy Number :**
  - **Hosts :** (E.coli)
  - **Suppliers :** (Invitrogen)
  - **Misc. Comments :**
  - **Parents :** (pSL300 from pSLJ10 from pBluescript KS+)
  - **Siblings :** ()
  - **Descendents :** ()
- 

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170-20  
3264  
1736

3264  
1618

3264  
1618  
1736  
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118



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Nucleotide

Entrez	PubMed	Nucleotide	Protein	Genome	Structure	PMC	Taxonomy	Books
Search		Nucleotide	for		Go		Clear	
Limits		Preview/Index		History		Clipboard		Details
Display	default	Show	20	Send to	File	Get Subsequence		Fe

☐ 1: U14656. Bovine herpesviru...[gi:641976]

Links

LOCUS BHU14656 1614 bp DNA linear VRL 25-MAY-1995  
DEFINITION Bovine herpesvirus type 5 glycoprotein D (gD) gene, complete cds.  
ACCESSION U14656  
VERSION U14656.1 GI:641976  
KEYWORDS .  
SOURCE Bovine herpesvirus 5  
ORGANISM Bovine herpesvirus 5  
Viruses; dsDNA viruses, no RNA stage; Herpesviridae;  
Alphaherpesvirinae.  
REFERENCE 1 (bases 1 to 1614)  
AUTHORS Abdelmagid,O.Y., Minocha,H.C., Collins,J.K. and Chowdhury,S.I.  
TITLE Fine mapping of bovine herpesvirus-1 (BHV-1) glycoprotein D (gD)  
neutralizing epitopes by type-specific monoclonal antibodies and  
sequence comparison with BHV-5 gD  
JOURNAL Virology 206 (1), 242-253 (1995)  
MEDLINE 95133156  
PUBMED 7530392  
REFERENCE 2 (bases 1 to 1614)  
AUTHORS Chowdhury,S.I.  
TITLE Direct Submission  
JOURNAL Submitted (14-SEP-1994) Shafiqul I. Chowdhury, Pathology and  
Microbiology, College of Veterinary Medicine, Kansas State  
University, 1800 Denison Avenue, Manhattan, KS 66506, USA  
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#### ORIGIN

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### Definition of cosmid :

A hybrid plasmid that contains cos sites at each end. Cos sites are recognized during head filling of lambda phages. Cosmids are useful for cloning large segments of foreign DNA (up to 50 kb).

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Would you have liked more information?	<input type="button" value="No"/> <input type="button" value="Submit"/>

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